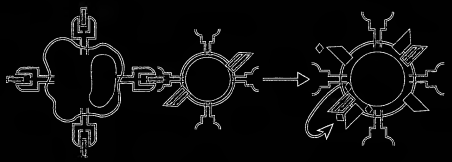
SECOND EDITION

CELLULAR AND MOLECULAR IMMUNOLOGY



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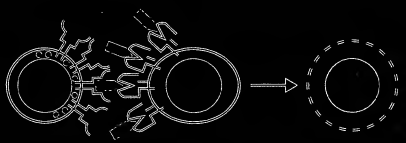

SECOND EDITION

CELLULAR AND MOLECULAR IMMUNOLOGY

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ter, many of the effector functions of immunoglobulins are mediated by the Fc portions of the molecule.

Different results are obtained when the proteolytic enzyme pepsin is used instead of papain to cleave rabbit IgG molecules (Fig. 3–8). In this case, under limiting conditions of enzyme concentrations and time, proteolysis is restricted to the carboxy terminus of the hinge region near the $C_\gamma2$ domain such that the antigen-binding fragment of IgG retains the hinge and the interchain disulfide bonds. Fab fragments containing the heavy chain hinge are called Fab′; when the interchain disulfide bonds are intact, the two Fab′ fragments remain associated in a form called **F(ab′)$_2$.** The Fc fragment is often extensively degraded and does not survive proteolysis by pepsin. Fab and F(ab′)$_2$ are often useful as experimental tools because they can bind to antigens without activating Fc-dependent effector mechanisms.

These proteolysis experiments are not readily extended to other antibody isotypes such as IgM. In fact, they are not even applicable to all IgG molecules in many species other than rabbit. However, the basic organization of the Ig molecule that Porter deduced from his studies of rabbit IgG is common to all Ig molecules of all isotypes and of all species. These features may be summarized as follows:

1. Each V_LV_H pairing forms an independent antigen-binding site. Thus, all monomeric IgG molecules have two separate antigen-binding sites, and secreted pentameric IgM molecules have ten separate antigen-binding sites (see Figs. 3–2 and 3–6).

2. The structure of the hinge region (or lack of one in certain isotypes) sterically determines how many binding sites of a single antibody molecule can simultaneously interact with antigen molecules, e.g., on a cell surface.

3. The Fc portion of an antibody molecule is spatially distinct from and functions independently of the antigen-binding site formed by the Fab regions. Since Fc regions activate immune effector functions, the kinds of effector functions activated by a particular Ig molecule are largely independent of the specificity for antigen and instead depend primarily on the isotype of the antibody.

ANTIBODY BINDING OF ANTIGENS

In the preceding sections, we have developed a general description of the structure of antibody molecules. Now we will turn to a more detailed discussion of the structural basis and physicochemical characteristics of antigen binding.

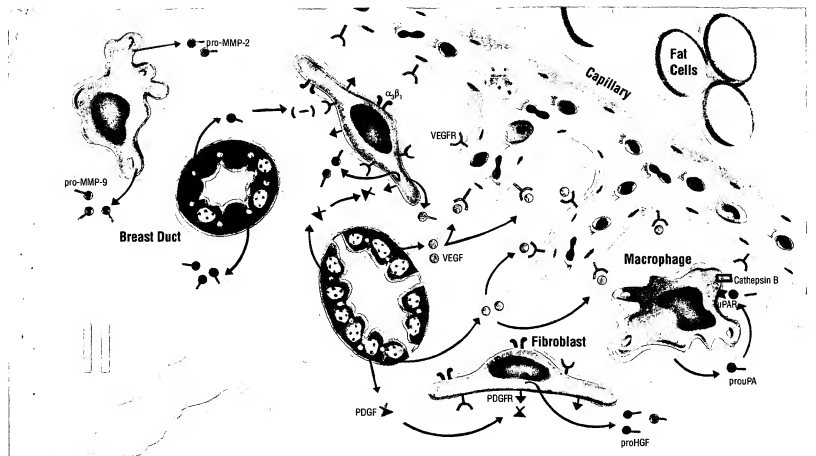
Structural Aspects of Biologic Antigens

An **antigen** can be defined as any substance that may be specifically bound by an antibody molecule. This differs from the original (historical) definition of antigen as a molecule that generates an antibody. We now know that almost every kind of biologic molecule, including simple intermediary metabolites, sugars, lipids, autacoids, and hormones as well as macromolecules such as complex carbohydrates, phospholipids, nucleic acids, and proteins, can serve as antigens. However, only macromolecules can initiate lymphocyte activation necessary for an antibody response. Molecules that generate immune responses are called **immunogens.** (Although technically less precise, the more inclusive term "antigens" is still commonly used to refer to "immunogens.") In order to generate antibodies specific for small molecules, immunologists commonly attach such small molecules to macromolecules before immunization. In this system, the small molecule is called a **hapten** and the macromolecule, usually a foreign protein, is called a **carrier.** The hapten-carrier complex, unlike free hapten, can act as an immunogen.

In general, macromolecules are much bigger than the antigen-binding region of an antibody molecule. Therefore, an antibody binds to only a specific portion of the macromolecule, called a **determinant,** or **epitope.** These two words are synonymous and are used interchangeably throughout the book. A hapten may be thought of as an exogenous determinant that is attached to a macromolecule.

Macromolecules typically contain multiple determinants, each of which, by definition, can be bound by an antibody. In some cases, the determinants are spatially well separated, and two individual antibody molecules can be bound to the same antigen molecule without influencing each other; such determinants are said to be non-overlapping. In other cases, the first antibody bound to an antigen may sterically interfere with the binding of the second, and the determinants of the antigen are said to be overlapping. In rarer cases, binding of the first antibody may cause a conformational change in the structure of the antigen, influencing the binding of the second antibody by means other than steric hindrance. Such interactions are called allosteric effects.

In the case of phospholipids or of complex carbohydrates, the antigenic determinants are entirely a function of the covalent structure of the macromolecule. However, in the case of nucleic acids, and even more so in the case of proteins, the non-covalent folding of the macromolecule may also contribute to the formation of determinants. In proteins, epitopes formed by adjacent amino acid residues in the covalent sequence are called **linear determinants** (Fig. 3–9). It is estimated that, in a protein antigen, the size of the linear determinant that forms contacts with specific antibody is about six amino acids long. Linear determinants may be accessible to antibodies in the native folded protein if they appear on the surface or in a region of extended conformation. More often, linear determinants may be inaccessible in the native conformation and appear only when the protein is denatured. In contrast, **conformational determinants** are formed by amino acid residues from separated portions of the linear amino acid sequence that are spatially juxtaposed only upon folding (Fig. 3–9). In theory, denatured proteins could transiently give rise to conformational



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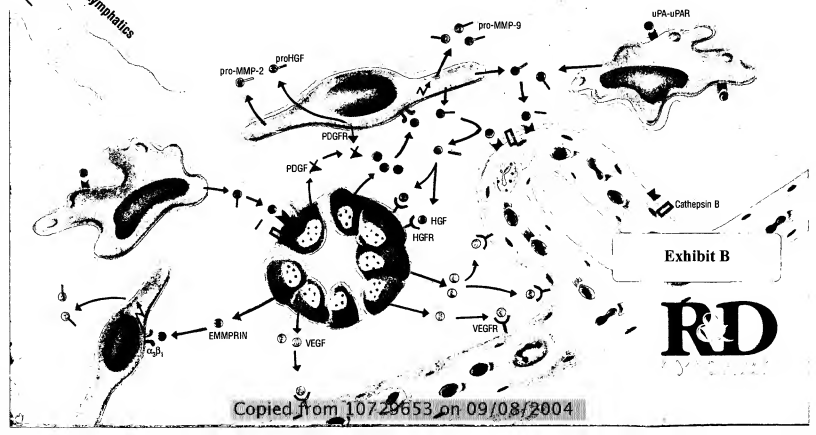

Exhibit B

R&D

Antibodies

R&D Systems' polyclonal and monoclonal antibodies to cytokines, receptors and related molecules are raised against purified proteins and affinity-purified using either protein A, G, or antigen columns. Each antibody has been tested for neutralization of bioactivity as well as for use in direct ELISA and western blot applications. Many antibodies have been labeled and tested for use in flow cytometry applications and for building ELISAs. For more information on matched antibody pairs for ELISA, refer to the section on ELISA development.

Antigen affinity purified polyclonal antibodies are at least 10-fold more potent than protein G affinity purified antibodies. The use of analyte-specific affinity chromatography almost eliminates non-specific immunoglobulin. The polyclonal antibody pool recognizes more than one antigenic epitope giving a stronger staining signal than monoclonal antibodies. Many affinity purified antibodies have been tested by scientists world-wide for cytokine-specific immunostaining.

Antibodies To Cytokines & Related Molecules

anti-Bcl-x
polyclonal antibody

Catalog #	**AF800**
Size/Price	**50 µg/$190**
Form	Antigen affinity-purified, 0.2 µm filtered solution in PBS and NaN_3, lyophilized
Type	Mouse/human Bcl-x specific rabbit IgG
Immunogen	Synthetic peptide corresponding to amino acids 49 - 68 of the Bcl-x sequence
Specificity	Selected for its ability to react with human and mouse Bcl-x.
Western Blot	An antibody concentration of 0.3 µg/mL is recommended.
Immuno-precipitation	3 µg of antibody per immunoprecipitation of Bcl-x from 7 x 10^5 A431 cells is recommended.

anti-Bcl-x_L
monoclonal antibody

Catalog #	**2300-MC-100**
Size/Price	**100 µg/$230**
Form	0.2 µm filtered solution in PBS, liquid
Type	Mouse IgG_{2A}, clone # YTH-2H12
Immunogen	Synthetic peptide corresponding to amino acids 3 to 14 of the human Bcl-x_L sequence. This epitope is also present in Bcl-X_S, a variant of an alternative RNA splicing.
Specificity	Selected for its ability to recognize human, mouse and rat Bcl-x_L.
Applications	See product insert for performance specifications.

anti-human BDNF
polyclonal antibody

Catalog #	**AF248**
Size/Price	**100 µg/$340**
Form	Antigen affinity-purified, 0.2 µm filtered solution in PBS, lyophilized
Type	Human BDNF specific chicken IgG
Immunogen	*Sf* 21-expressed recombinant human BDNF
Specificity	Selected for its ability to recognize rhBDNF.
Applications	See product insert for performance specifications.

anti-human BDNF
monoclonal antibody

Catalog #	**MAB248**
Size/Price	**500 µg/$280**
Form	0.2 µm filtered solution in PBS, lyophilized
Type	Mouse IgG_1, clone # 35928.11
Immunogen	*Sf* 21-expressed recombinant human BDNF
Specificity	Selected for its ability to neutralize the bioactivity of rhBDNF.
Neutralization	5 - 15 µg/mL will neutralize 50% of the bioactivity due to 2.5 ng/mL of rhBDNF.
ELISA	0.5 - 1 µg/mL will detect <12.5 ng/well of rhBDNF.
Western Blot	1 - 2 µg/mL, with the appropriate secondary reagents, will allow visualization of approximately 300 ng/lane of rhBDNF under both non-reducing and reducing conditions.

anti-CPP32 polyclonal antibody

Catalog #	**AF-605-NA**
Size/Price	**100 µg/$230**
Form	Antigen affinity-purified, 0.2 µm filtered solution in PBS and NaN_3, lyophilized
Type	CPP32 specific goat IgG
Immunogen	Recombinant human CPP32 zymogen
Specificity	Selected for its ability to react with intact human CPP32 and with CPP32 fragments of ~20, 18, and 16 kDa that are produced during apoptosis.
Western Blot	0.5 µg/mL, with the appropriate secondary reagents, is recommended.
Immuno-precipitation	4 µg of antibody per immunoprecipitation of human CPP32 from 1 - 2 x 10^6 cells is recommended.

anti-human CTLA-4 polyclonal antibody

Catalog #	**AF-386-PB**
Size/Price	**100 µg/$340**
Form	Antigen affinity-purified, 0.2 µm filtered solution in PBS, lyophilized
Type	Human CTLA-4 specific goat IgG
Immunogen	*Sf*21-expressed recombinant human CTLA-4, extracellular domain
Specificity	Selected for its ability to detect rhCTLA-4 in direct ELISA and western blot applications.
ELISA	0.5 - 1 µg/mL will detect <0.16 ng/well of rhCTLA-4.
Western Blot	0.1 - 0.2 µg/mL, with the appropriate secondary reagents, will allow visualization of approximately 5 ng/lane of rhCTLA-4 under both non-reducing and reducing conditions.

Also Available Biotinylated (Catalog # BAF386, Size 50 µg, Price $365)

anti-mouse CRG-2/IP-10 polyclonal antibody

Catalog #	**AF-466-NA**
Size/Price	**100 µg/$340**
Form	Antigen affinity-purified, 0.2 µm filtered solution in PBS, lyophilized
Type	Mouse CRG-2 specific goat IgG
Immunogen	*E. coli*-expressed recombinant mouse CRG-2
Specificity	Selected for its ability to neutralize the bioactivity of rmCRG-2. In direct ELISA and western blot applications, this antibody shows <10% cross-reactivity with rhIP-10.
Neutralization	4 - 12 µg/mL will neutralize 50% of the bioactivity due to 200 ng/mL of rmCRG-2.
ELISA	0.5 - 1 µg/mL will detect <0.16 ng/well of rmCRG-2.
Western Blot	0.1 - 0.2 µg/mL, with the appropriate secondary reagents, will allow visualization of approximately 5 ng/lane of rmCRG-2 under both non-reducing and reducing conditions.

Also Available Biotinylated (Catalog # BAF466, Size 50 µg, Price $365)

anti-mouse CTLA-4 polyclonal antibody

Catalog #	**AF476**
Size/Price	**100 µg/$340**
Form	Antigen affinity-purified, 0.2 µm filtered solution in PBS, lyophilized
Type	Mouse CTLA-4 specific goat IgG
Immunogen	NSO-expressed recombinant mouse CTLA-4, extracellular domain
Specificity	Selected for its ability to recognize rmCTLA-4.
Applications	See product insert for performance specifications.

Also Available Biotinylated (Catalog # BAF476, Size 50 µg, Price $365)

labeled anti-human CXCR-5 (BLR-1) monoclonal antibody

Catalog #	FAB190B	FAB190P
Size/Price	100 Tests/$205	100 Tests/$230
Label	Biotin	Phycoerythrin
Form	1 mL in a HEPES buffered saline containing 0.5% BSA and 0.1% NaN_3.	
Type	Mouse IgG_{2B}, clone # 51505.111	
Immunogen	CXCR-5 transfected NSO mouse myeloma cells	
Specificity	Selected for its ability to react by FACS analysis with human CXCR-5 transfectants. This antibody does not cross-react with CXCR-2, CXCR-3, and CXCR-4 transfectants.	
Flow Cytometry	10 µL, with the appropriate secondary reagents, can be used to stain $1x10^5$ cells for flow cytometry applications.	

anti-human Cytochrome *c* monoclonal antibody

Catalog #	MAB898
Size/Price	100 µg/$200
Form	0.2 µm filtered solution in PBS, lyophilized
Type	Mouse IgG_{2A}, clone # 2B5F8
Immunogen	Equine Cytochrome *c*
Specificity	Selected for its ability to recognize human Cytochrome *c*.
Applications	See product insert for performance specifications.

anti-Cytochrome *c* monoclonal antibody

Catalog #	MAB897
Size/Price	100 µg/$200
Form	0.2 µm filtered solution in PBS, lyophilized
Type	Mouse IgG_{2B}, clone # 7H8.2C12
Immunogen	Pigeon Cytochrome *c*
Specificity	Selected for its ability to recognize human or mouse Cytochrome *c*.
Applications	See product insert for performance specifications.

anti-Cytochrome *c* monoclonal antibody

Catalog #	MAB899
Size/Price	100 µg/$200
Form	0.2 µm filtered solution in PBS, lyophilized
Type	Mouse IgG_{2A}, clone # 2.7D5
Immunogen	Pigeon Cytochrome *c*
Specificity	Selected for its ability to recognize human or mouse Cytochrome *c*.
Applications	See product insert for performance specifications.